Accuracy of Transforming Growth Factor-Beta in Serum and Synovial Fluid as Rheumatoid Arthritis Prognostic Factors

Ali J. Almousawy[1] and Mayyada F. Darweesh[1]

[1]*University of Kufa, Department of Microbiology, Faculty of Science, Kufa, Iraq*

KEYWORDS

Rheumatoid arthritis (RA), TGF-β1, mild stage and aggressive stage

ABSTRACT

Background: Rheumatoid arthritis (RA) An autoimmunity of the joints which is now seen as a major public health concern. Therefore, finding new biomarkers is crucial to improve RA sufferers' quality of life. Several cytokines increased the inflammatory cascade which linked to pathophysiology of RA. This investigation aimed to determine the validity of transforming growth factor-beta-1 (TGF-β1) in synovial fluid and serum as prognostic factor and which one is better for rheumatoid arthritis disease follow up.
Material and Methods: In Al-Saddar Medical City Rheumatology Unit in the Najaf governorate, 80 patients were included in case-control research. These patients were identified by specialized who classified the patients according to stages of disease into mild stage (stage 1 and 2) and aggressive (stage 3 and 4 who had knee effusion and synovial fluid separation). Venous blood samples (3ml) drawn from patients and controls were transferred to a gel tube for serum separation that used to measure the TGF-β1 level in accordance (BT LAB/China), RF level and anti-CCP level by ELISA assay. knee synovial fluid was collected from RA patients in aggressive stage and from patient suffering from knee trauma as control group which included 18 patients not have RA.
The result demonstrated that Females and aged individuals in AL-NAJAF province more incidence with RA disease. TGF-β1 serum level increased significantly with disease severity and positive correlated with ACCP autoantibody, according to ROC prognosis analysis AUC of TGF-β1 serum level was excellent 0.9 with 100% sensitivity and specificity. While ROC analysis for TGF-β1 synovial fluid was 0.8 with 77.8% sensitivity and 86.7% specificity
Conclusion: Using ROC analysis, which supported good TGF level in both serum and synovial fluid diagnosis accuracy and enabled more sensitive and precise discrimination in RA disease. Instead of using synovial fluid separation as a diagnostic for excellent prognosis in RA disease, TGF-β1 and ACCP blood levels can help patients avoid discomfort and stress.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune joint disease marked by bilateral symmetric polyarthritis that in long-term untreated instances results in joint erosions, destruction and disability, persistent inflammation generates pro-inflammatory cytokines and autoantibodies (Abdullah et al., 2024). Through the influx of inflammatory cells into the tissues, a complex network of cytokines involved in the pathogenesis of RA primarily affect the synovium, resulting in joint destruction and synovitis., several cytokines increased the inflammatory cascade lead to progressive RA (Zhang and Dhalla.,2024).

Together with other forms of autoantibodies, anti-citrullinated protein antibodies (ACCP) gather in the joints and are highly specific for RA. They have been linked to the development of the disease. then attract immune cells to release chemokines and activate the complement system as well as enhanced immune response which can exacerbate chronic inflammation and bone loss (Mastellos et al.,2024). There are four stages of RA which is: early or stage 1, moderate-stage 2, severe stage 3 and end- stage 4. Symptoms may not appear until moderate stage (Holers et al.,2022).

TGF-β1 an essential regulatory pleiotropic cytokine play key role like control immune response, wound healing, cell proliferation, and differentiation. TGF-β1 has proinflammatory and immunological effector properties in addition to its primary immune system role of suppressing lymphocyte proliferation and differentiation, TGF-β can induce Th17 differentiation in humans and mice with proinflammatory cytokines present, specifically IL-6 or IL-21 (Honing et al.,2024). Both IL-12 and It has been proven that TGF-β has an important function in the pathophysiology of SLE. (Hassan et al.,2022). (Lee et al., 2024) confirmed role of TGF-β1 in onset and course of RA disease.

The levels of cytokines and chemokines may indicate the inflammatory state in joints, even if there is no clear biological marker for the diagnosis and prognosis of RA. A number of research have examined the viability of biomarker analysis in serum and synovial fluids in arthritis. which possible provide the answer to both the correct diagnosis and effective treatment of arthritis. So, this early-phase clinical trial attempted to provide a preliminary estimate of TGF-β level in sera and synovial fluid and possible correlation with ACCP as prognosis RA disease.

2. Methodology

Patients and control characterization:

A case-control study design used on 120 participants, comprising 40 healthy control group members ranging between 20-65 and 80 patients with rheumatoid arthritis, spanning both sexes and various age and stages of disease, who were taken from the Rheumatology Unit in Al-Sadder Teaching Hospital in AL-Najaf Al-Ashraf state. The patients were examined by a specialist physician and diagnosed as having rheumatoid arthritis based on clinical, radiological, and serological parameters according to 2010 ACR/EULAR criteria from October 2023 till the end of March 2024.

Included and Excluded criteria: All RA This research consisted of patients with both sexes and ranged in age. Patients with co-occurring autoimmune diseases, chronic illnesses, and other forms of arthritis were excluded from this study, although those with RA. Also, no intraarticular injections administered to the knee joint in the past 3 months

Sample collection: Three ml of vinous blood of all subjects were separated using a gel tube for serum separation which was then used to measure the RF, ACCP autoantibodies according to (Alegria, Germany) and TGF-β1 level was analyzed in serum and synovial fluid samples accordance to Bioassay Technology Laboratory (BT LAB/China). Synovial fluids were obtained from knee joints of RA patients and 18 patients suffering from knee trauma but hadn't RA as control group

Ethical approval: The study was carried out in compliance with moral guidelines derived from the Helsinki Declaration. Prior to sampling, the patient's permission was obtained. Using document number 1100 from the faculty of science, the local ethics committee accepted the study protocol, subject information, and permission form on 11/3/2024.

Statistical analysis

Data were expressed as means ±S.D. Statistical analyses were performed through SPSS 20 followed by an independent T-test, one-way ANOVA, ROC curve and P-value. The Receiver operating characteristic (ROC) curve was made to assign the best cutoff point that amplifies the sensitivity, specificity, and to identify its cutoff point that differentiates among patients and healthy control.

3. Result and Discussion

Results

Demographical Distribution of Rheumatoid Arthritis patients and control subjects

The current findings appear that RA patients included more females 64(80%) than male 16(20%) and 40 apparently healthy with female 31 (77.5%) and male 9 (22.5%) as shown in table (1). According to means age of RA patients 50.6±16.5 years while healthy subjects recorded 42.5±9.24 years. All patients were RF positive compare to negative results in all healthy groups. In this study patients classified as mild stage that included (stage 1 and 2) and aggressive stage (patients in stage 3 and 4)

Table (1): Case -control design of Rheumatoid arthritis patients and healthy group

variable	control group	Patients
	N(%)	N(%)
Sex;		

Male	9 (22.5%)	16 (20%)
Female	31 (77.5%)	64 (80%)
Stages of disease;		
Mild-stage	-	30(37.5%)
aggressive-stage	-	50(62.5%)
RF autoantibody;		
	negative (-)	positive (+)
Age;		
Age means	42.5 ± 9.24	50.6 ± 16.5
Age range	20-65	18-76
<40 (n%)	8 (20%)	17 (21.25%)
40-59 (n%)	19 (47.5 %)	38 (47.5 %)
≥ 60 (n%)	13(32.5 %)	25 (31.25%)
Total	**40 (100%)**	**80 (100%)**

These results consist with local study in Rheumatology Unit in Al-Sadder Teaching Hospital in AL-Najaf province by (Faihan and Darweesh,20 24) they showed higher frequency of RA in women (90%) than in men with elevated incidence in patients aged 40-59 years with mean age 47.75±14.20. (Zhang *et al.*,2024) they indicated that the age group of 45 years and older had a strikingly high prevalence of RA and suggests that RA is more common in older age groups.

Recent epidemiological research indicated that women often have RA disease beginning symptoms around middle age or menopause, but males usually suffer from disease onset symptoms later in life and are more likely to test positive for RF and have higher levels of ACCP (Antonijevic *et al.*,2024). This study similar to a local study by (Khafaji *et al.*,2024) revealed that the mean age of RA patients was 47.7±10.9 years (20 - 70 years). 5.09% of these patients were men, and 90.9% of them were women. and clarify that sex hormones, which have intricate impacts on the immune system, may be the cause of the high incidence in women. Sex hormones can lead to risk, as evidenced by the increased frequency of autoimmunity in women. Progesterone promotes the production of T-helper2, while estrogen is known to have a biphasic dosage impact, with lower levels enhancing and higher levels inhibiting certain immunological activity (Th2) cells with proliferation of the T-helper1 (Th1) cells (Elserougy *et al.*,2023).

The results observed that all patients were RF positive while all apparently healthy group were negative results for RF, this similar to study by (Mode *et al*.,2022). who founded in meta-analysis study that 7517 patients with RA were RF positive by latex test with highest sensitivity (68.6%, 95% CI 66.2% to 71.0%). (Hammam *et al*.,2024) illustrated that RA patients have positive RF and high RF levels are linked to extra-articular symptoms and more severe articular disease, particularly when combined with ACCP autoantibody. Also, (Riccardi *et al*.,2022) observed that RF and ACCP highly elevated in RA patients compare to healthy group and confirmed that presence of these autoantibodies serves as a predictive factor for the onset of rheumatoid arthritis in undifferentiated arthritis patients as well as the development of a more severe disease progression, marked by increased joint erosions over time.

Evaluation the level of ACCP in RA patients and control group as prognostic factor:

These results revealed higher significance ACCP levels (170.123 ± 155.94 U/ml) for RA patients in compare to control group (10.6 ± 3.59U/ml) at P value= 0.0001. Among patients, ACCP serum level was highly increased in aggressive-stage vs mild- stage of disease (647.93 ± 187.94 vs 45.81 ± 6.66 U/ml) P≤ 0.0001 as shown in figure (1). The appropriate cut-off value of ACCP was 22.45U/ml which

had 100% sensitivity and specificity with excellent AUC 1.000 as shown in figure (2).

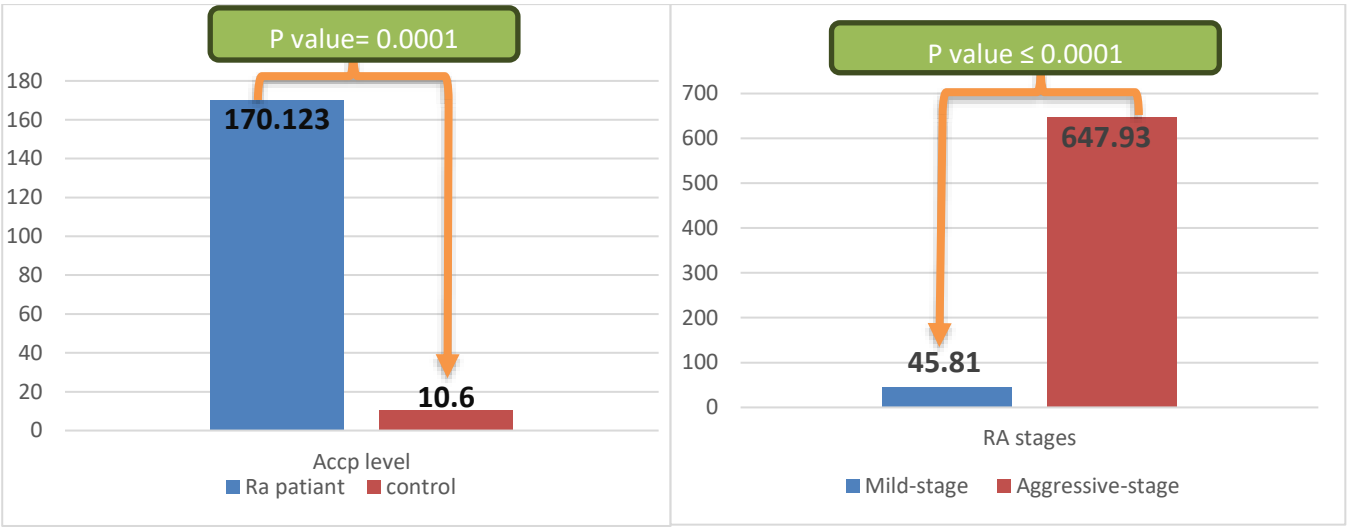
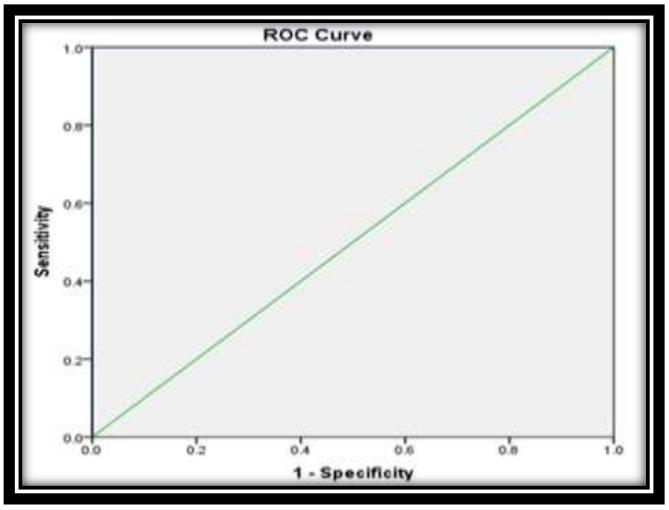


Figure (1): A-level of ACCP in patients and healthy. B- ACCP level in RA according to stages of disease



AUC	1.000
P-value	**0.0001**
cut-off	**22.45U/ml**
sensitivity	**100%**
specificity	**100%**

Figure (2): ROC curve analysis of ACCP in RA patient's vs control.

The present study found that blood levels of ACCP were higher in RA patients than in controls, which is consistent with results from a local study (Faihan and Darweesh,2024) who observed that ACCP antibody high level in the serum of RA patients versus healthy control with high specificity and sensitivity and correlated with progressive of disease ,the previous researchers in Baghdad, Iraq (Hassoon *et al.*,2020) who revealed that the mean of Anti-CCP in controls was (0.244 ± 0.004), while in RA patients was 0.504 ± 0.006 (P-value = 0.0001). And in Najaf , a study elucidate that Anti-CCP levels were particularly higher level in the RA group compared to the control group at p<00001 (Jassim *et al.*,2023) There are variances in the results across different studies, and these variations in sensitivity could be caused by a variety of factors, including different cut-off values, variations in serum dilution, variations in detection techniques between reports, variations in the unit of expression, variations in the duration of the disease, and other clinical characteristics within the tested groups.

The appearance of Anti-CCP antibodies indicates pathophysiological properties and a potential involvement to on-going immune activation (Turcinov *et al.*, 2023). Since ACPAs are thought to have an independent effect on bone remodeling and to perform an important part in bone mass loss during the pre-clinical stage of RA, individuals who had these antibodies did not exhibit synovial inflammation (Pignatti *et al.*,2024).

Estimation the level of TGF-β1 in RA patients and healthy controls

The findings show that blood TGF-β1 levels in RA patients were considerably ($p < 0.0001$) higher than control group (762.1±86.4 versus 316.1±91.3 ng/ml , as illustrated in figure (3) Also, this figure showed that TGF-β1 serum level was highly increased among patients in late- stages (909.7 ± 14.9 ng/ml) compare to early-stages $P \leq 0.0001$(3-B) .ROC curve demonstrate that the optimal cut-off value of TGF-β1 was 540.9 ng/ml, with 97.5% sensitivity, 100% specificity, AUC 0.998 (95%CI 0.993 - 1.000) as showed in figure (4)

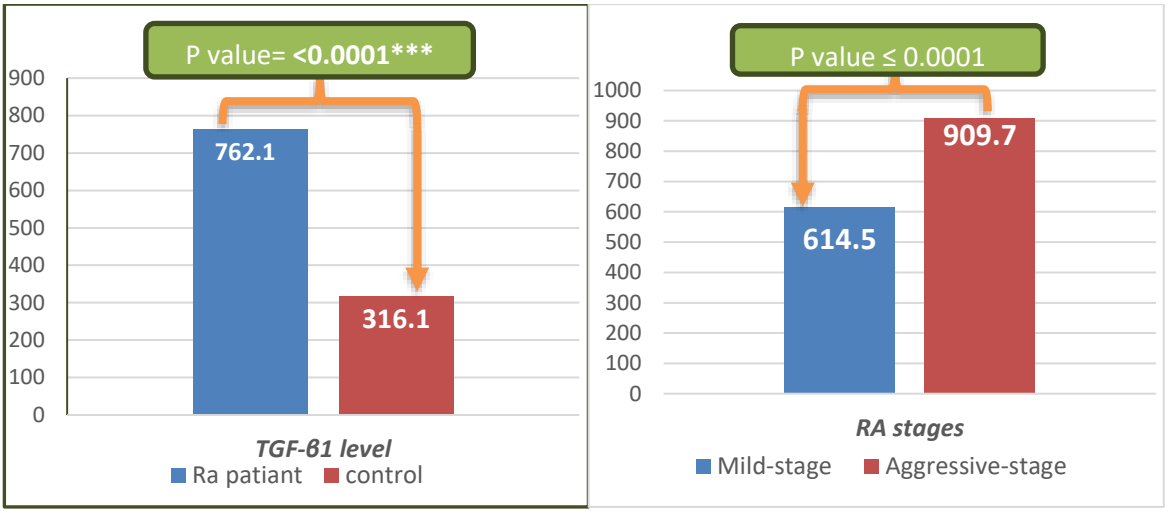
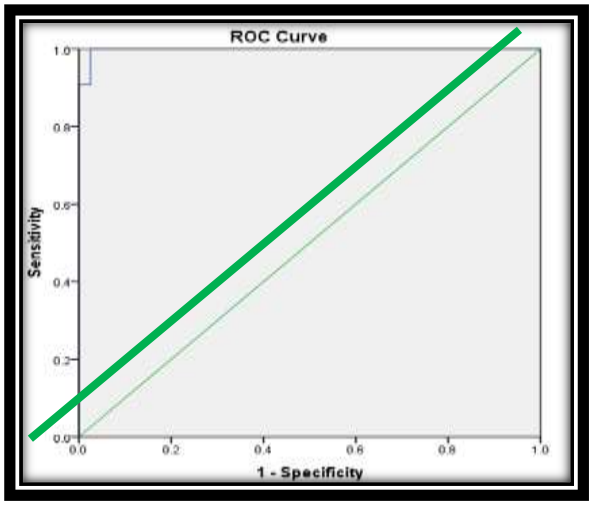


Figure (3) A- TGF-β1 level in RA and healthy



AUC	**0.998**
P-value	**≤ 0.0001**
cut-off	**540.9 ng/ml**
sensitivity	**97.5%**
specificity	**100%**

Figure (4): ROC curve analysis as diagnostic sensitivity and specificity of RA patient's serum TGF-β1.

The current study demonstrated a significant difference of TGF-β1 level between RA group and control group. (Aarts *et al.*,2022) They demonstrated that, in compared with healthy controls, RA patients obtaining DMARD treatment and those receiving a new diagnosis both had significantly higher plasma levels of TGF-β1. This in line with (Paradowska-Gorycka *et al.*,2022) they demonstrated that TGF-β1 serum level increased in RA patients than healthy control group with statistically significant differences. Similar to study by (Mieliauskaite *et al.*, 2023) they confirmed that blood levels of TGF-β1 were higher in RA patients ($P < 0.05$) compared to healthy controls and observed that TGF-β1 pathogenetic involvement in the development of disease. RA patients had higher levels of TGF-β1 found in their plasma, indicating there might be an effect on the occurrence of RA. (Piramoon *et al.*, 2024).A study found that TGF-β1 blood levels were higher in RA patients ($P < 0.05$) when compared to healthy controls, demonstrating that serum TGF-β1 levels may be an indicator of continuing autoimmune inflammation that is linked with damage to joints in RA (Mieliauskaite *et al.*, 2019).

According to some studies, the reason for the increased TGF-β levels might be because the protein is produced as the massive, inactive precursor known as the latency-associated peptide (LAP), which is the precursor of TGF-β. The inactive form of TGF-β, known as LAP, is a latent form that is unable to attach to its receptor. The intracellular protease separates LAP-1 from the remaining protein to produce physiologically active TGF-β. (Coutts *et al*.,2001Since poor cell responsiveness to TGF-β, particularly because of defective TβR activities, is likely the cause of autoimmune inflammation, increased TGF-β synthesis is a compensatory response. (Abaas et al.,2024). These results consist with (Brown *et al*.,2024) they observed elevated TGF-β1 concentration in the early stages of RA causing destructive in cartilage with progressive the disease. TGF-β and TGF-βRI overexpression in rheumatoid synovial fibroblasts is associated with clinical indicators of disease activity, demonstrating a connection between TGF-β and inflammation. (Guo *et al.,*2024). (Gonzalo-Gil *et al.,*2013) they shown that TGF-β is induced in the joints at the time of arthritis onset and increases afterwards . This result consists with (Thielen *et al.,*2022) who observed that patients during severe or advanced stages of RA, the levels of TGF-β highly increased compared to earlier stages. In the early stages of RA, TGF-β levels are typically elevated which contributes to the recruitment and activation T cells and macrophages to the affected joints (Tian *et al*.,2024). In the later stages of RA, when joint damage and deformities become more severe, TGF-β levels may increase again (Rajdan *et al.,*2024). Increased synthesis of TGF-β and IL-12 with their correlation with disease severity have also been reported in diabetic foot ulcer (Mohsin *et al.,*2024).

Evaluation the level of TGF-B1 in synovial fluid of RA patients and healthy control:

The current study revealed the mean TGF-β1 level in synovial fluid of RA patients and control groups (1091.1±37.9U/ml and 398.4±22.3U/ml) respectively with high significance (P value <0.0001***) as shown in figure (5).

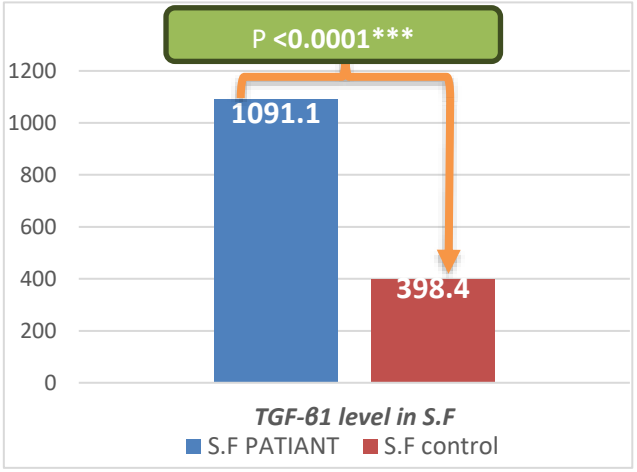
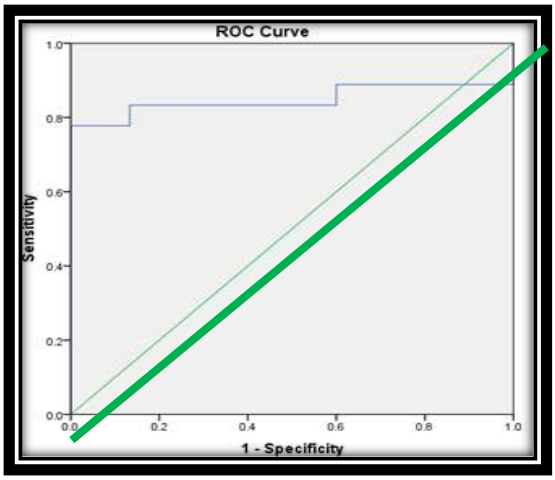


Figure (5) A- TGF-β1 in synovial fluid of RA patient and healthy control



AUC	0.848
P-value	**0.00****
cut-off	**496.1**
sensitivity	**77.8 %**
specificity	**86.7%**

Figure (5) B-ROC curve of TGF-β1

This study found elevated SF level of TGF-β1 this compatible with previous study by (Shibata *et al.*,2024) Research has shown that TGF-β1 is highly expressed in RA patients' joints and produced in substantial amounts in synovial fluid. It has also been found that synovial cells may generate TGF-β1 both in vivo and in vitro. The concentration of TGF-β1 was significantly elevated in SF of RA patients than normal SF and confirmed that TGF-β1 induces differentiation of synovial fibroblasts to α-SMA-positive myofibroblasts which play a key role in RA-associated synovial inflammation. Also, (Ollewagen *et al.*,2021) revealed that TGF-β1 highly increased in RA patients especially in pre- treated patients compare to healthy control group and RA treated patients and explain that TGF-β1 plays a pivotal role in fibrosis and pathogenesis associated with RA. In line with finding (Danielpour *et al.*,2024) who found that increased TGF-β level in SF of RA patients leads to the activation and proliferation of synovial fibroblasts, which contribute to the development of pannus formation and joint destruction. the levels of TGF-β are elevated in the synovial fluid and tissues of patients with early-stage RA (Kotschenreuther *et al.*,2022).(Sisto *et al.*,2021) mention that TGF-β elevated in synovial tissue and synovial fluids of RA patients, so it governs the activity of fibroblasts and may have a role in the pathophysiology of RA. Also, (Wang *et al.*,2019), mentioned that the RA group had higher expressions of TGF-β, IL-6, and TNF-α than the control group.

4. Conclusion and future scope

strong correlation between TGF- and ACCP serum level that correlated with disease severity. Using ROC analysis, which supported good TGF level in both serum and synovial fluid diagnosis accuracy and enabled more sensitive and precise discrimination in RA disease .Instead of using synovial fluid separation as a diagnostic for excellent prognosis in RA disease, TGF-β1 and ACCP blood levels can used as prognostic factor which help patients to avoid discomfort and stress.

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